VOLUME 3

BASF Biocides Ltd

PRODUCT CHEMISTRY

MYACIDE AS TECHNICAL

EPA reg. no. 33753-3

(Additional supplier)

Data requirements

40 CFR 158.150

Guidelines

OPPTS No. 830.1800
(Analytical Methods)

Author

Mrs Glynis Cierech

Date

May 2003

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for information contained in this study on the basis of its falling within the scope of FIFRA 10(d)(1)(A),(B), or (C).

Company:	BASF Biocides Ltd
Company agent:	Mrs Glynis Cierech
Title	Registration Officer
Signature:	G. Cierech
Date	June 9, 2003

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The information in this volume is not required to meet the GLP requirements specified in 40 CFR Part 160.

Submitter/Sponsor/Study Director: Mrs Glynis Cierech

Company: BASF Biocides Ltd

Signature: G. Cierech

Date: June 9, 2003

MYACIDE AS TECHNICAL (New producer – [redacted]

OPPTS 830.1800 Analytical Method

BASF Aktiengesellschaft

BASF Aktiengesellscaft – 67056 Ludwigshafen

BASF

Spezialchemikalien
Speciality Chemicals

Myacide AS Technical

Analytical Procedures April 15, 2003

Examine the sample for general appearance.

Infrared Spectrum.

Either:

Evenly disperse approximately 10% sample/KBr mixture, (usually 25mg of the sample to 250mg Potassium Bromide). Use the sample cup method.

Or:

Evenly disperse approximately 1% sample/KBr mixture, (usually 2mg of the sample to 250mg Potassium Bromide). Prepare a pressed disc.

Record the infra red spectrum over the range 2000 to 400 cm^{-1}. Compare the spectrum obtained with that, which has been prepared from a reference standard.

pH of a 1% solution at 20°C

Determine using a suitable pH meter.

Water.

Determine on 2g as described in the European Pharmacopoeia under "Semi Micro Determination of Water".

Determination of related compounds by high pressure liquid chromatography.

These working conditions are given for guidance only and may require modification for optimum performance.

Mobile Phase. Pipette 100ml of far UV grade acetonitrile and 10ml of 10% v/v orthophosphoric acid into a 2 litre volumetric flask, make up to volume with water. Do not filter the mobile phase, degas using a sonic bath.

Test Solution. Dissolve 10mg of tris-(hydroxymethyl)nitromethane, 2-methyl-2-nitropropan-1,3-diol, 2-nitroethanol and sodium bromide in 100 ml of mobile phase, mix. Weigh about 100mg of bronopol assay reference standard, add 1ml of the above solution and dilute to 50 ml with mobile phase.

Preparation of sample solution. Dissolve 100mg (± 10mg) of sample in 50 ml of mobile phase.

The chromatographic conditions are as follows:

Column	A Phenomenex $C_{18}(2)$ Column, 15 cm long and 4.5mm internal diameter or equivalent.
Column temperature:	35°C
Mobile phase flow rate:	1.0 ml per minute*.
Wavelength for detection:	214 nm.
Volume injected:	5 µl.
Chromatographic run time:	45 minutes.

* adjusted to give an elution time of about 7 minutes for bronopol.

System suitability. Check that the relative retention times of the test solution impurities are similar to those give below, and that each impurity is easily detectable. The sodium bromide and tris-(hyroxymethyl)nitromethane should be almost baseline resolved, if not, check and adjust the mobile phase pH to 3 using dilute sodium hydroxide solution. This may assist in the separation of the peak. The tris-(hydroxymethyl)nitromethane and 2-nitroethanol should be baseline resolved.

Determination. Inject the sample solution. Measure the areas under the peaks on the chromatogram by integration, using the correction factors given below, any unknown peaks should be estimated by assuming that they have the same response as bronopol and a correction factor of 1, and calculate the percentage of each impurity.

Compound	Relative Retention Time	Correction factor (CF)
Sodium Bromide	0.28	1.1
Tris-(hydroxymethyl)nitromethane	0.30	0.6
2-Nitroethanol	0.34	0.4
Nitromethane	0.40	0.3
2-Methyl-2-nitropropan-1,3-diol	0.48	0.6
Bronopol	1.00	1.0
2-Bromo-2-nitroethanol	1.40	0.7

$$\% \text{ Impurity} = \frac{\text{area of impurity peak x CF x 100}}{\text{sum of corrected areas of all peaks}}$$

Assay by high pressure liquid chromatography

These working conditions are given for guidance only and may require modification for optimum performance.

Mobile Phase. Pipette 100ml of far UV grade acetonitrile and 10ml of 10% v/v orthophosphoric acid into a 2 litre volumetric flask, make up to volume with water. Do not filter the mobile phase, degas using a sonic bath.

The chromatographic conditions are as follows:

Column	A Phenomenex $C_{18}(2)$ Column, 15 cm long and 4.5mm internal diameter or equivalent.
Column temperature:	35°C
Mobile phase flow rate:	1.0 ml per minute*.
Wavelength for detection:	214 nm.
Volume injected:	5 µl.
Chromatographic run time:	45 minutes.

* adjusted to give an elution time of about 7 minutes for bronopol.

Internal Standard Solution. 0.1% of 3-acetamidophenol in mobile phase.

Determination.

Standard preparation. Accurately weigh about 0.1g of bronopol assay reference standard into a suitable bottle. Add 10.0ml of internal standard solution, dilute to 100ml with mobile phase. Mix

Prepare a further "check" standard, accurately weigh 0.12g of bronopol assay reference standard, and make up as above.

Sample preparation. Accurately weigh 0.1g of sample into a suitable bottle. Add 10.0ml of internal standard solution, dilute to 100ml with mobile phase. Mix.

It may be necessary to condition the column with injections of the standard solution. Chromatograph the standard, "check" standard and sample in duplicate, and determine the ratio of the bronopol peak area to that of the 2-acetamidophenol in each case.
Where F is % purity of reference material.

The approximate relative retention times of the peaks of interest are as follows:

Compound	Relative Retention Time
Bronopol	0.44
3-Acetimidophenol	1.00

$$\% \text{ Bronopol} = \frac{\text{ratio for sample x weight of standard x F}}{\text{ratio for standard x weight of sample}}$$

Where F is % purity of reference material